Meeting of the States Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction

24 July 2014

English only

2014 Meeting
Geneva, 1-5 December 2014

Meeting of Experts
Geneva, 4-8 August 2014
Item 6 of the provisional agenda
Standing agenda item: review of developments in the field of science and technology related to the Convention

Advances in science and technology: Understanding pathogenicity and virulence

Submitted by the United States of America

I. Introduction

1. States Parties are halfway through the current intersessional programme, the first for which science and technology (S&T) relevant to the Convention is a standing agenda item. Fittingly, the S&T agenda item is an experiment, wherein Parties are exploring effective mechanisms for keeping up to date on relevant S&T advances. The United States considers this an important goal. To achieve it, Parties would benefit from information from recent scientific papers on **what** the relevant scientific knowledge and technologies are, **how** they are routinely used in laboratories, and the potential significance of recent discoveries and trends. To this end, we review below specific examples of research on pathogenicity and virulence from the past year. Empowered with this information, States Parties can more easily keep up to date, recognize truly game-changing scientific knowledge and technologies and, most importantly, grasp the challenges they pose to the implementation of this Convention. The United States of America delegation seeks feedback from States Parties regarding the clarity, relevance, and usefulness of this paper, as well as suggestions for improvement.

2. With regard to S&T developments, States Parties are perhaps most interested in their implications for the BWC. Acknowledging this, we discuss in section II the potential implications posed by recent research on pathogenicity and virulence. Section III seeks to define and explain the key concepts of pathogenicity, virulence and host-pathogen interactions in a manner that is technically correct but accessible to non-specialists. Sections IV and V each comprise two examples of recent scientific papers representing the two major types of pathogens: viruses and bacteria. Key technical terms appear in italics and are defined in the text or in footnotes.

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II. Implications

3. Perhaps the most significant trend in this area in recent years is our steadily advancing appreciation of the importance of host-pathogen interactions. That is, rather than understanding disease as simply a function of infection by a pathogen, studies have increasingly led to an understanding that disease is the result of a complex interaction between the pathogen and the host organism. This helps us to understand why one microbe may cause disease and another, very similar one does not produce any symptoms, or why a pathogen may make some people ill and not others.

4. Scientific research will continue to produce knowledge about host-pathogen interactions. New technologies combined with this knowledge will enable quicker and more detailed analyses of how these interactions lead to disease. Taken together, these S&T developments can reveal key interactions between host and pathogen that can be blocked in order to treat or prevent disease. For example, discovery of *virulence factors*[1] can guide vaccine and drug development. It is highly desirable to have vaccines and drugs that specifically block the effects of virulence factors, because they can ameliorate symptoms while fighting the pathogen.

5. As more is learned about host-pathogen interactions, it will become easier to more accurately predict the presence and effects of virulence factors in emerging pathogens based on sequence similarities or antibody cross-reactivity, as was done by researchers in the case of botulinum toxin Type H (Example 4 in Section V below). In some instances, for example with methicillin-resistant *Staphylococcus aureus* (MRSA), this predictive capability is already well advanced.[2] When outbreaks from new or re-emerging pathogens occur, knowledge gained from studies on the mechanisms and evolution of *pathogenesis*[3] should enable more rapid responses and development of countermeasures.

6. A capability to make predictions about the outcomes of host-pathogen interactions is inherently dual use knowledge. In a positive sense, such knowledge would improve and hasten development of vaccines, therapeutics, and diagnostics with increased specificity for virulence factors. Such knowledge could also enable mitigation of the host immune response, turning it up or down to minimize host damage. On the other hand, a predictive capability could theoretically be applied to the design of more virulent pathogens. While the topic of "designer pathogens" is disquieting, it is important to note that there are significant knowledge gaps – as well as technical hurdles – to their production. A very good example is the current difficulty in making predictions about which genes in a pathogen contribute not only to virulence, but also to viability, transmissibility to other hosts, and stability in the environment. While the *genomes*[4] of many pathogens have been sequenced, the functions of individual genes are frequently unknown or incompletely understood – though continued research is expected to fill these knowledge gaps over time.

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[1] Virulence factors are genetic, biochemical or structural features of a pathogen that enable disease (discussed in Section III).

[2] Laabei M, Recker M, Rudkin JK et al. (9 Apr 2014) Predicting the virulence of MRSA from its genome sequence. *Genome Research* 24:839-849, http://genome.cshlp.org/content/24/5/839.

[3] Pathogenesis refers to the chain of events that lead to disease.

[4] A genome is the complete set of genetic material within an organism or microorganism, including its genes.

III. Pathogenicity and Virulence are Determined by Pathogen and Host Factors

7. *Pathogenicity* and *virulence* are closely related terms that are sometimes used interchangeably. In the case of microorganisms, pathogenicity is the capacity to cause disease in a host. Virulence is the **degree** of damage that a pathogenic microorganism, or *pathogen*, causes in a host.

8. Certain attributes of pathogens play roles in determining the presence and extent of disease; these attributes are called *virulence factors*. However, a pathogen's virulence is determined not only by what virulence factors it contains, but is dependent on factors within the host as well. These *host factors* include genetic background, physical condition, and the status of the host immune system. For example, immune status is an important host factor; some microorganisms cause disease in immunocompromised[5] humans but not in healthy humans. Genetic background is another example: the human immunodeficiency virus (HIV) cannot establish chronic infection in humans possessing mutations in a single gene called CCR-5. In many, if not most cases, host factors that affect virulence remain unknown; one common example is herpes simplex virus 1, which for unknown reasons causes cold sores in some humans but not others, despite being highly contagious. Therefore, pathogenicity and virulence are determined by both pathogen and host factors.[6]

IV. Viral Pathogenesis and Virulence

Box 1: Two major viral mechanisms of pathogenesis

1. **By directly causing death of infected cells**: To complete their life cycles, viruses must gain entry into host cells, multiply inside those cells, and release new viruses to spread to other cells. To multiply, viruses hijack host cell machinery and deprive that cell of energy and materials necessary for survival. Release of newly replicated virus ultimately kills the host cell. If not stopped by an effective antiviral immune response or therapeutic agent, cell death may proceed to destroy tissues and eventually organs. This destruction can take place quickly (as when cells in the brain are destroyed by infection with encephalitis viruses) or slowly (as the liver is destroyed by persistent infection with hepatitis viruses).

2. **By stimulating host immune responses that destroy cells and tissues**: Some viruses cause hosts to mount strong immune responses that lead to cell death and tissue destruction more severe than damage caused by viral infection itself. For example, some influenza viruses quickly elicit severe inflammation (the first stage of an immune response) in the lungs that damages delicate cells essential for breathing. Even if such an immune response were effective at destroying the virus, the host may die from lung damage caused by the immune response itself.

9. **Example 1: Recent studies on virulence factors of influenza virus provide an example of current approaches to studying viral pathogenesis.** In June of this year,

[5] An immunocompromised host is one with an immune system that is impaired; this impairment may be caused by drugs, genetic deficiency, certain cancers, or chronic infections like HIV.

[6] For a thorough discussion of this topic, see Casadevall and Porofski's 2001 paper Host-Pathogen Interactions: The Attributes of Virulence, available at www.iid.montana.edu/faculty/hardy/documents/virulence_attributes_Casadevall.pdf.

Kawaoka and colleagues published a paper[7] in the journal *Cell Host & Microbe* that described experiments using *recombinant*[8] avian influenza viruses generated in the laboratory. Genes from known influenza viruses were chosen for their high degree of similarity with genes from the 1918 pandemic influenza virus and assembled into a new "1918-like" avian influenza virus. This 1918-like virus was used to infect ferrets – a commonly used animal model for the *in vivo*[9] study of influenza virus. The virulence of the 1918-like virus was studied by measuring weight loss and mortality of infected ferrets. (The sicker an animal becomes, the less likely it is to eat; therefore, weight loss is used as a proxy for degree of illness.) The 1918-like virus caused ferrets to lose an amount of weight intermediate between that caused by an unaltered strain of avian influenza virus and by the 1918 virus, leading to the conclusion that the artificial 1918-like strain is more pathogenic than unaltered avian influenza virus and more similar to the original 1918 virus. They also examined the lungs of infected ferrets – both the original 1918 virus and the 1918-like virus spread throughout lung tissues and damaged those tissues, unlike the unaltered avian virus. These and further experiments prompted the researchers to conclude that pandemic risk exists, since the genes similar to those from the 1918 virus circulate naturally and have the potential for enhancing virulence were they to recombine into a 1918-like virus in nature.

10. **Example 2: Recent review of molecular mechanisms by which varicella zoster virus causes two diseases in humans.** In a different approach published in February of this year, Zerboni and colleagues summarized models of the pathogenesis of varicella zoster virus (VZV), which causes chickenpox[10]. Like the herpesvirus that causes cold sores, VZV produces *latent* infection, in which the viruses lie dormant in nerve cells (neurons) and can later reactivate to cause another infection. Unlike, for example, influenza viruses, which only infect respiratory tissues, the life cycle of VZV plays out across a variety of tissues: first immune cells (called T cells) in the tonsils, then via the blood to the skin (where it causes pustules, or pox), then into neurons and sometimes back again to the skin (where it causes shingles, a painful skin rash). Scientists have developed a picture of how VZV manipulates three host cell types – T cells, skin cells and neurons – by exhaustively cataloging the effects that viral proteins exert on host proteins. To discover the effects of these proteins, scientists mutated the VZV genes encoding the proteins in order to make the proteins nonfunctional. Using this method, scientists discovered several mechanisms by which VZV proteins prolong infection, including: (i) promoting survival of infected T cells and skin cells, (ii) enabling VZV to go unrecognized by the immune system, and (iii) inducing "gene silencing" that enables the virus to go dormant in neurons.

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[7] Watanabe T, Zhong G, Russell CA et al. (11 Jun 2014) Circulating Avian Influenza Viruses Closely Related to the 1918 Virus Have Pandemic Potential, *Cell Host & Microbe* 15: 692-705, http://www.cell.com/cell-host-microbe/abstract/S1931-3128(14)00163-2.

[8] A recombinant virus comprises genes or gene fragments assembled from different sources.

[9] The term *in vivo*, Latin for "within the living," means occurring within a complete living system. Most commonly, *in vivo* experiments are experiments conducted in animals.

[10] Zerboni L, Sen N, Olivier SL et al. (10 Feb 2014) Molecular mechanisms of varicella zoster virus pathogenesis. *Nature Reviews Microbiology* 12:197-210, http://www.nature.com/nrmicro/journal/v12/n3/full/nrmicro3215.html.

V. Bacterial Pathogenesis and Virulence

Box 2: Two major bacterial mechanisms of pathogenesis

1. **By invasion of host cells and tissues**: Invasion requires contacting and sticking to cells, secreting invasive substances (called invasins) and avoiding the immune system while bacteria multiply. For example, tuberculosis, gangrene, and streptococcal infections result when the causative bacteria invade host cells and tissues.

2. **By producing toxins**: Some pathogenic bacteria secrete exotoxins (such as botulinum, diphtheria or cholera toxins) that act at a distance from the infected site in the body. Endotoxins are parts of bacterial cells to which hosts may have severe immune reactions when the bacteria die and disintegrate in the host. For example, fever and shock result when endotoxins within pathogenic strains of *E. coli* bacteria are released into the bloodstream – a disease state known as sepsis.

11. **Example 3: Recent studies of virulence factors contributing to persistent infections of *Mycobacterium tuberculosis*.** Advances in microbiology and genome sequencing technologies are increasingly applied along with classical approaches to answer basic questions in bacterial pathogenesis. For example, in studying the invasive pathogen *Mycobacterium tuberculosis* (Mtb), Cunningham-Bussel and colleagues sought to identify potential virulence factors in the pathogen's *genome* in order to understand the ability of Mtb to survive for long periods in human lungs.[11] This research suggested Mtb enzymes as virulence factors that allow survival within host cells under different *in vivo* conditions. Targeting these enzymes with drug candidates may provide an effective way to interfere with Mtb growth and persistence in host cells.

12. **Example 4: Recent characterization of a newly discovered botulinum toxin.** A new botulinum toxin (Type H) from a strain of *Clostridium botulinum* was characterized by Barash and Arnon[12] using a combination of classical immunology, *genome* sequencing and animal studies.[13] Immunological approaches indicated that antibodies to known botulinum toxin (types A through G) did not react with the new toxin; genome sequencing revealed its similarities and differences to other botulinum toxins; and animal studies demonstrated its extreme toxicity as well as the potential for vaccine development. The evidence presented by the researchers suggested that existing therapeutics and vaccines would not be effective against the novel Type H toxin.

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[11] Cunningham-Bussel A, Zhang T, Nathan CF (5 Nov 2013) Nitrite produced by *Mycobacterium tuberculosis* in human macrophages in physiologic oxygen impacts bacterial ATP consumption and gene expression, *PNAS* 110(45): E4256-E4265, http://www.pnas.org/content/110/45/E4256.full

[12] Barash, JR and Arnon, SS. (7 Oct 2013) A Novel Strain of Clostridium botulinum That Produces Type B and Type H Botulinum Toxins Journal of Infectious Diseases 209:183-191, http://jid.oxfordjournals.org/content/209/2/183.

[13] Hooper et al., 2014. J Infect Dis. 209:167 (DOI: 10.1093/infdis/jit528); Dover et al. 2014. 209:192-202 (DOI: 10.1093/infdis/jit450).

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Item 8 of the provisional agenda
Biennial item: how to strengthen implementation of Article VII, including consideration of detailed procedures and mechanisms for the provision of assistance and cooperation by States Parties

Focusing Efforts to Strengthen Article VII: A proposed agenda for international cooperation and assistance in preparing for and responding to biological incidents

Submitted by the United States of America

1. At the 2011 BWC Review Conference, the States Parties to the Biological and Toxins Weapons Convention (BWC) agreed to discuss "How to strengthen implementation of Article VII" at the 2014 and 2015 intersessional meetings. Article VII, which has never been invoked, commits all States Parties to "provide or support assistance … to any Party to the Convention which so requests, if the Security Council decides that such Party has been exposed to danger as a result of violation of the Convention."

2. As we have noted in previous national papers (BWC/MSP/2013/MPX/WP.6 and BWC/MSP/2013/WP.2), timely response to a biological incident is critical in order to prevent morbidity and mortality, and in mounting an effective early response, the origin of the event - be it deliberate, accidental or natural - may not yet be known. Thus efforts to strengthen implementation of Article VII should focus, first and foremost, on how to ensure efficient, effective response to an outbreak at the earliest possible point, and ensuring that transition to formal activation of Article VII provisions is seamless and complementary to any ongoing public health or animal health response. This is consistent with the importance placed by the Review Conference on "ensuring that efforts undertaken are effective irrespective of whether a disease outbreak is naturally occurring or deliberately caused, and cover diseases and toxins that could harm humans, animals, plant or the environment." Although a deliberate biological weapons incident poses additional challenges (for example, evidentiary, chain-of-custody, and security considerations), it is undergirded by public/animal health and emergency response capabilities, on the part of both donor and recipient, that are largely required for both intentional and naturally-occurring events.

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3. Since the 2011 BWC Review Conference, the United States and other governments have sought to stimulate discussion on how to improve preparedness for and response to an Article VII event. Several States Parties have submitted working papers and hosted side events to highlight national efforts to plan for and organize response to biological incidents; showcase the work being done by an array of international organizations, both in humanitarian disaster response as well as efforts directly in support of biological incidents; and identify a series of challenges to and opportunities for the provision and acceptance of international assistance during public health emergencies. These papers and events have described lessons learned from natural disaster response in the United States., other States' efforts to address impediments to international assistance, and the planning processes and challenges experienced by international organizations in providing assistance during public health emergencies.

4. This paper advocates that BWC member States agree on an agenda to strengthen implementation of Article VII by selecting issue areas where the BWC forum can complement other global efforts and make real progress towards improving preparedness and response to biological incidents. Other international agreements, initiatives, and organizations are committed to enhancing preparedness and response efforts for humanitarian disasters and public health emergencies. Multiple international organizations regularly engage in response to humanitarian disasters, including the United Nations Children's Fund (UNICEF), World Food Programme (WFP), United Nations High Commissioner for Refugees (UNHCR), Food and Agricultural Organization (FAO), Office for the Coordination of Humanitarian Affairs (OCHA), International Organization for Migration (IOM), the World Health Organization (WHO), and the World Organization for Animal Health (OIE). These organizations work with the Red Cross and Red Crescent Societies, the International Committee of the Red Cross (ICRC), and a host of other nongovernmental organizations, all of which focus on a range of assistance related to humanitarian disaster response, including provision of medical care, food, and shelter. Additionally, the International Health Regulations (2005) obligate nations to develop capacity to respond to public health emergencies of international concern, and the new Global Health Security Agenda identifies building the capacity of all nations to respond to human/animal infectious disease events as a core purpose.

5. Given the range of activities and efforts related to preparedness and response, it is important for the BWC forum to capitalize on what has already has been accomplished by other international entities, to seek their advice and input on these issues, and to focus on a set of discrete issues that are not sufficiently addressed and that have direct bearing on BWC Parties' ability to provide and receive assistance in the event of a biological incident. To that end, we propose that States Parties focus on the following agenda for strengthening Article VII:

Public Health Emergency Medical Countermeasures (MCMs)

- Improve global access to MCMs during responses to public health emergencies through the development of bilateral, regional and global frameworks for the international deployment of medical countermeasures, consistent with the International Health Regulations (2005) and aligned with the objectives of the Global Health Security Agenda. These frameworks should address legal, regulatory, logistical, and funding challenges, including developing terms and conditions for deployment of MCMs that include liability arrangements; ensuring rapid regulatory approval to export, import and use MCMs in an emergency; and identifying or developing rapid funding options to purchase and transport MCMs and ancillary supplies (needles, syringes, etc.).

- Support WHO and other international partners in the development of adapted prequalification processes, or other regulatory mechanisms, to facilitate the import, distribution and use of emergency public health MCMs internationally.
- Explore bilateral, regional and multilateral opportunities for joint development and/or procurement of public health emergency MCMs, considering existing models such as Joint Procurement Agreement for MCMs in the European Union and the Pan American Health Organization Revolving Fund.
- Encourage review of the WHO Essential Medicine list to evaluate whether the MCMs on the list sufficiently address the need for WHO Member States to be prepared for known BW agents.

Public Health, Medical, and Veterinary Personnel

- Develop and strengthen bilateral, regional and global frameworks and tools to facilitate the international deployment of public health, medical, and veterinary personnel in response to international public and animal health emergencies. For example, this might be done by exploring ways to support ongoing initiatives such as the WHO Global Health Cluster's Foreign Medical Team (FMT) Working Group and WHO Regional Office efforts to develop guidelines, registries, and processes to facilitate the international deployment of medical and veterinary personnel during disasters. These frameworks and tools should address the legal, regulatory, logistical, and funding challenges to the international deployment of public health, medical, and veterinary personnel, including obtaining legal liability protections for responders, rapidly recognizing responders' professional licenses, and expediting customs and immigration procedures among other challenges.

Code on Rights and Responsibilities

- Develop a code for States Parties on rights and responsibilities for providing and receiving support in response to a biological emergency.

6. The proposed agenda for strengthening Article VII could begin this year, but to make real progress, States Parties would need to commit to a continued program of work that might extend over multiple years. Some of this work could be conducted within the BWC intersessional process, including discussion around improving access to medical countermeasures and how the BWC States Parties might support the FMT Initiative to strengthen Article VII. Other activities should be conducted in outside forums, such as reviewing the WHO Essential Medicine List, which will require participants to have specific expertise. States Parties should prioritize the development of internal capacities to respond efficiently and effectively to biological emergencies, while pursuing multilateral arrangements and agreements to facilitate external assistance during crises. The United States looks forward to working with States Parties and others to promote the development of capacities and frameworks to increase the speed and efficacy of international responses to biological incidents.

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Advances in science and technology: Evasion of the host immune response by pathogens

Submitted by the United Kingdom of Great Britain and Northern Ireland

Introduction

1. The topical scientific subject to be considered in 2014 is **"advances in the understanding of pathogenicity, virulence, toxicology, immunology and related issues"**. This is a particularly broad area covering a wide range of potentially relevant advances; however some developments reviewed under the standing agenda item on science and technology in the previous years of the current intersessional programme have implications also for our considerations this year. For example, in 2012 States Parties reviewed various enabling technologies, including those related to genomics and other '–omics', synthetic biology and systems biology, and agreed that their potential benefits to the Convention included, amongst other things, an improved understanding of the nature of disease and better healthcare technologies, such as improved, more efficient and economical vaccines, antibiotics and diagnostic systems. The United Kingdom paper on scientific and technological developments submitted for the Seventh Review Conference also highlighted that such technologies are useful tools in increasing the understanding of virulence mechanisms and host responses to pathogens and subsequently to development of new vaccines and therapeutics and improved diagnostics.[1] In the 2013 review, the advances recognised in relevant technologies for disease surveillance, detection, diagnosis and mitigation in many cases resulted from an increased understanding of the mechanisms of

[1] BWC/CONF.VII/INF.3/Add.1, pp 21-38.

disease and the host immunological response, as highlighted in the United Kingdom working paper on advances in vaccine development.[2]

2. Previous reviews also considered particular implications of such advances for the BTWC, including the potential for uses contrary to the provisions of the Convention, some of which will also be relevant to this year's topic; for example, increased capacity to manipulate pathogenicity, host specificity, transmissibility, resistance to drugs, or ability to overcome host immunity. In this context, States Parties have recognised that it is important to consider appropriate oversight measures to identify and manage associated risks in a way that will maximise the benefits of advances while minimising the risk of their application for prohibited purposes. The potential implications of advances in the understanding of pathogenicity, virulence, toxicology and immunology for other provisions of the Convention can also be recognised. These include those related to dealing with the consequences of a violation of the Convention, both in providing assistance to a State Party affected as a result and in any investigation process; and those relevant for capacity building and cooperation in the prevention of disease or for other peaceful purposes.

3. Given the immense range of potentially significant advances and the relevance of some topics previously reviewed, this Working Paper focuses only on one particular aspect: advances in the understanding of the strategies developed by pathogens to evade the host immune response and the consequent implications for the BTWC.

Evasion of host immune response: Background

4. In order for a microorganism to cause disease in a host organism, it must develop strategies to overcome the host mechanisms to defend against infection. Successful microbial pathogens have evolved strategies to evade both innate and adaptive immune systems[3], which may involve avoiding recognition by the immune system, interfering with its function, or degrading its components. There are many published reviews on this topic some of which outline general mechanisms used to subvert and exploit immune systems by diverse microbial pathogens,[4] and others which address mechanisms that have evolved in specific types of pathogens.[5] A fundamental expectation of studies in this field is that increased understanding of the molecular details of host-pathogen interactions and anti-immune systems will lead to a better understanding of the weaknesses in host defence systems and assist the development of vaccines and therapeutics. The following section provides a few recently published examples which reflect some of the different types of strategies utilised by pathogens in evasion of the host immune response.

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[2] BWC/MSP/3013/MX/WP.8.

[3] The innate immune system is the first line of host defence against invading pathogens and comprises cells and mechanisms that defend the host from infection in a non-specific manner, and activate the adaptive immune system. The adaptive immune system is specific to the invading pathogen and can provide long-lasting immunity.

[4] For example, Finlay, B.B & McFadden, G (2006) Anti-Immunology: Evasion of the Host Immune System by Bacterial and Viral Pathogens. Cell 124: 767-782.

[5] For example, Higgins, M.K & Carrington, M (2014) Sequence variation and structural conservation allows development of novel function and immune evasion in parasite surface protein families. Protein Science 23: 354-365; and Smith et al (2013) Vaccinia virus immune evasion: mechanisms, virulence and immunogenicity. Journal of General Virology 94: 2367-2392.

Evasion of host immune response: Some examples

5. Dengue virus has developed an ability to hide from host immune defences by avoiding recognition by components of the innate immune system. To do so it requires an enzyme known as MTase (or 2'-O-methyltransferase) to chemically modify its genetic material and escape detection, and thus survive, replicate and cause disease. Increased understanding of this mechanism has assisted progress towards effective vaccines and antivirals. For example, a recent study has reported a novel strategy in dengue vaccine development based on attenuated virus strains with a genetic mutation introduced to deactivate the MTase enzyme.[6] In these strains, the unmodified genetic material is immediately recognised by the host and triggers a response by innate immune factors. The viruses also elicit a strong adaptive immune response conferring protection on the host. Furthermore, it was found that that the MTase mutant dengue virus cannot infect the mosquito vector responsible for its transmission. This proof-of-concept study demonstrated that the attenuated viruses were stable, safe and immunogenic, and showed their potential as a safe, highly immunogenic, rationally-designed dengue vaccine approach.

6. Severe acute respiratory syndrome coronavirus (SARS-CoV) uses a different strategy to avoid detection in the host. It produces an enzyme called PLpro (or papain-like protease) which removes host cell proteins involved in triggering the innate immune response. This interferes with the host cell's signalling pathways and prevents it from alerting the immune system to the presence of the virus, thus allowing it to survive and replicate undetected. Increasing the understanding of such a mechanism will be of critical importance in providing a strategy for the development of vaccines and antivirals against SARS-CoV and other coronaviruses, such as MERS-CoV, for which there are currently no approved countermeasures. However, PLpro has another key function essential for viral replication, which has to be retained in a live vaccine strain to allow sufficient viral particles to be generated to trigger the immune response. Thus the goal of rational vaccine design would be to knock-out the system for evasion of the immune response whilst retaining the ability to replicate in host cells. A recent effort to understand the structural basis of PLpro activity has revealed key sites in the enzyme required for recognition and processing of the host cell proteins and elaborated its role in antagonism of the innate immune response.[7] This information enabled the creation of mutations in the enzyme to prevent interaction with the host cell proteins, while retaining the function for viral replication. Further work in this area is required, but this may represent a first step towards the development of a safe, live attenuated vaccine.

[6] Züst et al (2013) Rational design of a live attenuated dengue vaccine: 2'-O-methyltransferase mutants are highly attenuated and immunogenic in mice and macaques. PLoS Pathogens 9(8): e1003521. doi:10.1371/journalppat.1003521.

[7] Ratia et al (2014) Structural basis for the ubiquitin-linkage specificity and delSGylating activity of SARS-CoV papain-like protease. PLoS Pathogens 10(5): e1004113. doi:10.1371/journal.ppat.1004113.

7. Some pathogenic bacteria have an outer protective layer formed of capsular polysaccharides, which prevents them being recognised and destroyed by the host immune system. Thus the disruption of this layer could provide a strategy for therapeutic development, as highlighted in a recent publication.[8] This study focused on *Escherichia coli*, which forms the capsular polysaccharide inside the cell, then transports it through a membrane pore to the cell surface. The strategy involved using compounds that resembled the exported polysaccharide to block the pore and therefore disrupt formation of the protective outer layer. This allowed the bacteria to be recognised and killed by the host immune system, and hence could be key to the development of a new antibiotic class active against pathogens with similar mechanisms for capsule formation.

8. Some viral, bacterial, fungal and parasitic pathogens use a mechanism known as antigenic variation to avoid recognition by the adaptive immune response. The adaptive immune response relies on memory of previous exposure to specific antigens, which stimulated the generation of specific antibodies to target those antigens. Thus changing the molecules involved in promoting that response can circumvent the clearance of subsequent infection or sustain an on-going infection.[9] One of the most well-known and well-studied examples is influenza virus, where continuing to increase the understanding of the evolutionary patterns and molecular basis of antigenic change is important for vaccination and surveillance considerations. Recently published findings from research on the bacterium *Helicobacter pylori* (which causes ulcers in humans) suggest that it uses a strategy of rapid mutation during the initial infection phase that allows it to evade the immune system by quickly evolving. This results in elevated frequencies of changes in its surface proteins which may no longer be recognised by antibodies, thus allowing the bacteria to avoid the host immune response and to establish a chronic infection.[10] Trypanosomes and *Plasmodium* species, parasitic pathogens that cause sleeping sickness and malaria respectively, despite having very different life cycles have both evolved mechanisms to switch expression of variant surface proteins through antigenic variation to avoid antibody binding. Further studies will help understanding of the molecular details of the host-parasite interactions and guide the development of therapeutics to tackle parasitic disease.[11]

9. A key element of the innate immune response is the recognition of microbial components by receptors on the surface of host immune cells. When pathogens invade the host, proteins known as Toll-like receptors (TLRs) recognise pathogen-associated molecular patterns (PAMPs) and trigger a signalling pathway to activate an immune response. Within TLRs there is a conserved region known as the TIR domain which is critical for the protein-protein interactions involved in the signalling pathway. Recent work has identified a potentially novel evasion mechanism involving bacterial TIR domain proteins in a wide range of pathogenic bacteria, including *Salmonella enterica*, *Brucella*

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[8] Kong et al (2013) Single-molecule interrogation of a bacterial sugar transporter allows the discovery of an extracellular inhibitor. Nature Chemistry 5: 651-659.

[9] For review, see for example, Kotwal G. J & Kulkarni A.P (2013): Antigenic variation in microbial evasion of immune responses. eLS: doi: 10.1002/9780470015902.a0001207.pub3; and Lipsitch M & O'Hagan J.J (2007) Patterns of antigenic diversity and the mechanisms that maintain them. Journal of the Royal Society Interface **5**: 787-802.

[10] Linz et al (2014) A mutation burst during the acute phase of *Helicobacter pylori* infection in humans and rhesus macaques. Nature Communications 5: doi: 10.1038/ncomms5165.

[11] Higgins, M.K & Carrington, M (2014) Sequence variation and structural conservation allows development of novel function and immune evasion in parasite surface protein families. Protein Science 23: 354-365.

sp., E.coli and *Yersinia pestis*.[12] There is evidence to suggest that these interfere directly with the TLR signalling pathway and thus inhibit the activation of the innate immune system. Further understanding of the modes of action and the roles in virulence of these domains may help in developing strategies for novel countermeasures.

10. A further example of an evasion system involving interaction with receptors on the surface of host immune cells is a mechanism by which viruses such as influenza, West Nile and dengue activate a class of molecules, known as TAM receptors, which are central inhibitors of the innate immune response to pathogens. A recent study reported that a substance called phosphatidyl serine, found on the surface of enveloped viruses, bound to the immune cell surface proteins leading to disablement of the innate immune response.[13] Understanding of this mechanism may provide an attractive target for novel antiviral therapies which could block the virus's ability to activate TAM receptors and thus prevent interference with the innate immune system.

11. *Yersinia pestis* (the causative agent of plague) also disrupts the host immune system by interacting with a receptor protein on immune cells. *Y.pestis* requires the presence of a protein called the plasminogen activator protease (Pla) to survive within the lungs. It has recently been demonstrated that Pla activity degrades a molecule known as the Fas ligand (FasL) which is involved in the signalling process for a form of programmed cell death known as apoptosis. This interferes with the innate immune system and alters the host inflammatory response, enabling enhanced bacterial growth in the lungs, thus facilitating infection.[14] Pneumonic plague is fatal if untreated, and antibiotics are effective only if administered early, within the first 24 hours. Further understanding of the process by which *Y.pestis* evades the immune response may help in the development of effective medical countermeasures, for example by exploring the possibility that blocking Pla activity and thus restoring FasL signalling might give antibiotics more time to work.

12. A mechanism common to many bacteria involves the hijacking of host cells by the injection of microbial proteins in a so-called Type III secretion process, by which they exert a number of effects that assist their survival and block the host cell immune response. In some pathogens, one effect is to exploit the actin[15] polymerisation processes of the host cells to acquire actin tails that allow the bacteria to traffic rapidly through the host cell and from cell to cell.[16] Rapid cell-to-cell spread facilitates the avoidance of host innate and adaptive immune responses. This type of mechanism could provide a target for the development of antimicrobials with a novel mode of action, but further investigation is required to understand better the molecular processes involved.

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[12] See for example, Rana et al (2013) Bacterial TIR-containing proteins and host innate immune system evasion. Medical Microbiology & Immunology 202: 1-10; and Salcedo et al (2013) BtpB, a novel *Brucella* TIR-containing effector protein with immune modulatory functions. Frontiers in Cellular and Infection Microbiology 3: 1-13.

[13] Bhattacharyya et al (2013) Enveloped Viruses Disable Innate Immune Responses in Dendritic Cells by Direct Activation of TAM Receptors. Cell Host & Microbe 14: 136-147.

[14] Caulfield et al (2014) The Pla protease of *Yersinia pestis* degrades Fas ligand to manipulate host cell death and inflammation. Cell Host & Microbe 15: 424-434.

[15] Actin is a cellular protein involved in muscular contraction, cell motility and maintenance of cell shape.

[16] Allwood et al (2011) Strategies for intracellular survival of *Burkholderia pseudomallei*. Frontiers in Microbiology 2: 1-19.

13. A possible mechanism to evade the adaptive immune response that has recently been reported involves bacterial production of a broadly-reactive antibody-binding protein.[17] The protein, discovered in a *Mycoplasma* species that infects humans, was found to bind with high affinity to all types of human and non-human antibodies through attachment to conserved regions, thus blocking antibody-antigen binding. Since the interaction of specific antibodies with invading pathogens plays a key role in preventing infection, this represents an effective evasion strategy that interferes with the adaptive immune response. However further studies are required to elucidate this potential mechanism to defeat the antibody response, which if confirmed may offer a target for new antibacterial therapies. Given its apparent ability for broad-scope, high affinity binding to antibodies, another potential use for this protein is in the large scale purification of therapeutic antibodies.

14. Another mechanism recently described involves the degradation of a metabolite produced by immune cells called macrophages. These cells engulf invading pathogens inducing the production of a chemical called itaconate which interferes with bacterial metabolism. However some pathogens, including *Yersinia pestis,* can degrade itaconate, and three genes have been found to encode the enzymes responsible; these are crucial for the survival of some pathogens in macrophages.[18] In this case, as well as neutralising host defence mechanisms, the strategy also aids survival in the macrophage by breaking down itaconate into components that provide nutrients for the pathogen. The enzymes involved in this process might provide potential targets for the development of new antimicrobial agents.

Implications and conclusions

15. The above recent examples represent just a small proportion of the vast quantity of research on evasion of the host immune response by pathogens. The amount of effort in this area reflects its importance in the campaign to discover and develop novel strategies to address the challenges in defeating infectious disease. However, as with many fields in the life sciences, advances on this topic have the potential for uses contrary to the provisions of the Convention. Knowledge gained through research on host-pathogen interactions and mechanisms used to overcome the host immune response could also be exploited for harmful purposes, for example in designing novel biological weapons agents or engineering existing agents to increase their suitability for biological weapons use. It is thus important to ensure that appropriate oversight and governance strategies for such dual-use research are in place to minimise the risk of its use for prohibited purposes, without having adverse effects on crucial progress on infectious disease control. It is clear from recent discussions and debates on dual-use research issues that it is critical to undertake consideration of all the implications of proposed work, including those related to publication, at an early stage to ensure that both the potential benefits and risks are clearly balanced and articulated.

16. Advances in the understanding of evasion of the host immune response by pathogens have wide reaching potential benefits for the mitigation of infectious diseases and are highly relevant for the BTWC. The application of discoveries in this field to the development of vaccines and therapeutics has key implications for progress on the global response to infectious disease outbreaks, whether natural, accidental or deliberate. This is of relevance to the strengthening of Article VII, in providing assistance to any State Party exposed to danger as a result of a violation of the Convention, the biennial topic being

[17] Grover et al (2014) A structurally distinct human mycoplasma protein that generically blocks-antigen-antibody union. Science 343: 656-661.

[18] Sasikaran et al (2014) Bacterial itaconate degradation promotes pathogenicity. Nature Chemical Biology 10: 371-377.

addressed by States Parties in the remaining two years of the current intersessional programme. It is also a significant issue for activities related to Article X, in the development and application of scientific discoveries for the global prevention of disease, and for other peaceful purposes, and is thus relevant to the standing agenda item on cooperation and assistance.

17. The breadth of this year's topic again underlines the benefits of bringing in a range of knowledge and expertise from those more closely involved in relevant areas to assist us in considering the implications across all aspects of the Convention, and in assessing the need for effective action to manage potential benefits and risks. It also highlights the cross-over between the topics addressed in the review of scientific and technological advances over the years of the current intersessional programme, and thus the need to consider implications from previous understandings in our continuing reviews.

Meeting of the States Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction

25 July 2014

English only

2014 Meeting
Geneva, 1-5 December 2014

Meeting of Experts
Geneva, 4-8 August 2014
Item 8 of the provisional agenda
Biennial item: how to strengthen implementation of Article VII, including consideration of detailed procedures and mechanisms for the provision of assistance and cooperation by States Parties

Responding to a case of suspect biological weapons use: The command and control element at the scene

Submitted by the United Kingdom of Great Britain and Northern Ireland

Introduction

1. The first response to any case of suspect biological weapons use is very likely to be that of the State Party on whose territory an identifiable incident takes place; international assistance may appear on the scene shortly after the incident, or even some considerable time afterwards. In whichever scenario applies, an ability to organise, direct and contain such an incident is essential in ensuring an effective response in which humanitarian assistance can be provided promptly – primarily diagnostic, medical countermeasures and decontamination. The United Kingdom overall approach to preventing, preparing for and responding to a biological weapons attack was outlined in a working paper submitted to the Meeting of Experts in 2010.[1] A key issue highlighted was the need to address fully the command, control and coordination of multi-agency assets during an initial response and as the operation progresses. Command and control – the organisation, management and tasking of the response - especially in the initial stages when first responders arrive on the scene and are trying to determine the nature and scale of the problem, is thus a critical capability in ensuring early identification of the nature of the hazards and of the measures required to save lives.

2. This paper outlines some principles of command and control in this context based on United Kingdom national practice, which we believe might be of wider utility and which

[1] BWC/MSP/2010/MX/WP.7.

could be adopted or adapted by others to suit their own national circumstances. These principles may also be useful in thinking more generally about the organisation and provision of international assistance to a State Party exposed to danger as result of a violation of the Convention. This paper assumes a scenario in which there is an identifiable incident (or incidents) in a reasonably well defined area and where the initial presumption is that it could be CBRN in nature. In the case of a biological weapons attack, a deliberate release of agent may not always be obvious and may only become apparent gradually with no clear location of release on which to focus an immediate field response. In such cases, effective command and control at the strategic and tactical level remains essential for ensuring an effective response.

3. This working paper will focus only on *immediate* actions at the scene to give some idea of what is required. However, effective command and control must be maintained throughout the response to a biological weapons incident to avoid confusion amongst national emergency services and international assistance efforts arriving at the scene subsequently, and to prevent duplication of effort, misdirection or non-use of response capabilities and resources. Failure to do this effectively could make things worse, delay provision of assistance to those who require it, and prevent mitigation of the adverse health, economic and security consequences of the attack.

General principles

4. Clarity of purpose coupled with a clear definition of the various roles that are required for a first response to a suspect biological weapons incident are essential: delineation of responsibility for command and control *at the scene* of the initial incident is a fundamental requirement and this should reside in a single individual whose task will be to take overall operational command; to coordinate multi-agency activity and to decide upon and direct immediate action. In the United Kingdom there is a Joint Doctrine on the interoperability of the three emergency services for Emergency Response and Recovery when reacting to a major and complex incident.[2] This doctrine provides commanders at the scene and elsewhere with generic guidance on what actions they should undertake to deal with the incident. Three levels of command are outlined: operational, tactical and strategic and these are role not rank related. In the Joint Doctrine, command is defined as the exercise of vested authority that is associated with a role or rank within an organisation, to give direction in order to achieve defined objectives. Control is defined as the application of authority, combined with the capability to manage resources in order to achieve defined objectives.

Immediate challenges

5. It helps to break down the challenges into discrete well defined actions, which are largely sequential. In United Kingdom contingency planning nine such actions are central in the planning and execution of a response to a biological weapons incident. The tasks are:

- Command, control and coordination of emergency services and other resources.
- Mobilisation of responders to deal with the immediate consequences.
- Arrival at scene.

[2] See Joint Doctrine: the Interoperability Framework JESIP Joint Emergency Services Interoperability Programme http://www.jesip.org.uk/wp-content/uploads/2013/07/JESIP-Joint-Doctrine.pdf - annexes C, D, E (p.21-24) describe the roles and responsibilities for each level of command.

- Scene assessment.
- Scene management.
- Reconnaissance.
- Rescue and triage.
- Decontamination.
- Survivor management.

6. All activity must support the strategic intention of preserving and protecting lives, reducing the impact of the incident, informing the public and maintaining public confidence, and assisting an early return to normality. It must also be carried out in a way that ensures the health and safety of responders and safeguards the environment. It is also important to keep in mind the need to preserve evidence that could help in any investigation into source of the attack; there may be conflicts between the competing objectives of collecting and preserving evidence and providing humanitarian assistance that would need to be resolved.

Assessing and managing the situation

7. Three things must be done on first arrival at the scene of a suspect biological weapons attack: assess the situation; manage information; and manage the scene. Establishing what has happened requires collection and review of information as this provides the foundations on which to build an effective response. Relevant questions[3] include:

- What is already known about the incident?
- What information is available from first responders on the scene?
- What information exists from other sources such as eye-witnesses?
- What is the scale of the incident: are there any casualties?
- How many people appear to have been exposed? Are they showing any symptoms? If so, what symptoms are present?
- What are the release indicators?
    - Are there signs of blast damage to particular areas?
    - Is there any debris that may be from a dispersal device?
    - Are there suspicious pools of liquid or wet patches and splashes on the ground in normally dry conditions?
    - Are there any containers which appear unusual or out of place?
    - Are there any burst containers containing liquid or powder?
    - Are there any signs of vapour or mist, droplets or splashes not normally associated with that particular area?
    - Are ill, dead/dying people or animals present?

[3] Some of the information arising here might help identify the agent(s) used and therefore help better direct medical countermeasures, decontamination and quarantine efforts.

8. Identifying priorities for immediate action follows on from this along with assessing the immediate impact factors that could arise as a result of the incident, such as implications for critical infrastructure (water supplies, sewerage, power stations, hospitals, transport networks, food supplies) and populations downwind; this will help determine the steps required to mitigate the effects of the attack.

9. Keeping control of all the diverse and possibly conflicting strands of information that are likely to flow in large volumes in such situations is an essential task; there must be procedures and capabilities in place to ensure that it is collated, stored, and available and communicated to those who need it. This requires a command post to be established to serve as the central focal point in the field. Such a post helps the operational commander to coordinate a response and manage resources in support of tactical priorities. A command post needs to be staffed and equipped with appropriate information and communications technology (ICT). This post will also help manage the scene by ensuring that reports are handled promptly and instructions are directed appropriately; it will also provide a clear focal point for all those serving and supporting the response effort in the field. It will report developments and requirements to the tactical and strategic command levels.

Conclusion

10. This working paper emphasises the fundamental importance of effective command and control in the management and coordination of a response to a biological weapons attack and in containment of its effects. Many of the principles apply as much to an immediate national State Party response as to any international assistance teams arriving subsequently. The United Kingdom therefore suggests that the Meeting of Experts reaches a common understanding to this effect, with a view to the Meeting of States Parties making a recommendation for effective action as follows:

> "States Parties agree on the critical importance of effective command and control in the management and coordination of a response to a biological weapons attack and in the containment of its effects, and encourages those States Parties in a position to do so to assist other States Parties in the development of national capacities and capabilities and to place any offers of training or support on the assistance and cooperation database."